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Bacterial Contamination of the Swimming Pools in Shiraz, Iran; Relationship to Residual Chlorine and Other Determinants

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Abstract: The aims of this study were two fold; first to evaluate the degree of microbial contamination of the exposed and covered pools with selected groups of bacteria (major indices of water contamination) and second to assess the relationship between the degree of contamination and variables such as the mean value of residual chlorine, pH, temperature, etc. in the pools. Accordingly, numerous water samples were taken from all active pools and using the standard methods, were analyzed for bacteriologic and physicochemical properties. The data indicate that 51.3% of the total samples were contaminated with *Pseudomonas*. Furthermore, 16.6, 11 and 7% of the total samples were contaminated with *Escherichia coli*, fecal coliforms and streptococci, respectively. The mean value of residual chlorine in *Pseudomonas* contaminated samples was 0.45 mg L^{-1}. However, the corresponding value for non-contaminated samples was 1.052 mg L^{-1} and the difference was statistically significant ($p<0.02$). Similarly, 26.3% of the samples collected from the covered pools and 53.9% of those collected from exposed pools were contaminated with *Pseudomonas*. Statistical analysis of the data revealed that there was a significant difference ($p<0.02$) between the degree of contamination with *Pseudomonas* in exposed pools as compared to that of covered pools. Additionally, our data show that the source of water supply is also a major determinant of the degree of contamination. Surprisingly, public pools filled with well water were found to be less contaminated with different germs as compared to those filled with normal tap water. Moreover, the mean value of residual chlorine in *E. coli* contaminated samples was significantly different ($p<0.0008$) from that of non-contaminated samples. Exposed pools were found to be more contaminated with *E. coli* than covered pools. However, this difference could be attributed to a significant difference between the mean values of residual chlorine in these two different types of pools. Similar observations were made for contaminated and non-contaminated samples with fecal streptococci and coliforms. There was an inverse relationship between the number of coliforms and the mean value of residual chlorine in the pools. In conclusion, the observation that public pools in Shiraz, apart from a few exceptions, were generally contaminated with different germs, calls for a more strict supervision on the pools by the health authorities. In the absence of such supervision, contaminated pools continue to pose a significant risk to the health of swimmers.

Key words: Residual chlorine, swimming pools, water sanitation

INTRODUCTION

The protection of swimmers' health is of paramount importance in the use of swimming pools, as, the addition of debris like hair and secretory sebaceous substances from their body as well as pathogenic microorganisms from their respiratory, gastrointestinal and urogenital systems contaminate water. The lack of any antiseptic chemical compound, in sufficient concentration to control this contamination, prepares the ground for the prevalence of infectious diseases (Benenson, 1995).

In this regard, sanitation criteria for swimming pools have been devised which include water turbidity, temperature, residual chlorine, pH and the number of coliforms (ISIRI, 1998; APHA, 1998). The clarity of pool water has to be on such a level that a 15 cm diameter black plate could easily be seen on the deepest point of the white pool floor from a nine meter distance from either side (ISIRI, 1998). The pool water temperature must not be more than 25°C and the air temperature around pool should not be more than 5°C warmer and/or 1°C colder than the pool water (ISIRI, 1998). It is necessary to sample the pool water frequently to determine residual chlorine. According to conditions, the level of residual chlorine must be 0.6-1.0 mg L^{-1} (Kebabjian, 1995; ISIRI, 1998). In a bacteriologic perspective, the threshold limit for total number of coliforms should not exceed from 460 per 100 mL of water. The corresponding values for *E. coli*, fecal streptococci, mesophilic aerobic bacteria and *Pseudomonas aeroginosa* are 100, 100, 200 and zero,

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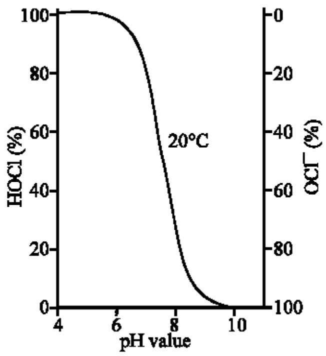

Fig. 1: Distribution of hypochlorous acid and hypochlorite ion at different pH values

respectively (ISIRI, 1998). Diseases transmitted to swimmers by the contaminated pool water could be divided into gastrointestinal, ophthalmic, otolaryngeal, dermal and parasitic infections. Although numerous chemical compounds like chlorine, bromine, iodine, ozone and on a more limited scale, physical disinfectants such as UV rays are used to disinfect pool waters; the most effective disinfectant in this respect is chlorine or its salts, which react with water and produce hypochlorous acid ($HOCl$) and hypochlorite ion (OCl^-), the microbicidal power of the former being much higher than the latter (Kebabjian, 1995). The concentration distribution of these two products under aqueous environments depends on pH; so that at low pH values, a larger percentage of free available chlorine is in $HOCl$ form and thus disinfection efficiency will be higher (Tchobanoglous and Burton, 1997). The disinfection efficiency of chlorine, however, is generally reduced since it is essential to keep nearly neutral pH in swimming pools (Fig. 1).

A number of studies have been carried out on the contamination of swimming pools. The results of a study on whirlpools in Finland and Sweden in 1995 revealed that 41% of swimming pool water samples were infected with amoebas (Vesaluma and Kalso, 1995). In the same year, an epidemic of infection with adenoviruses prevailed among swimmers in one of the southern Greek towns. The viral agent was rapidly diagnosed using PCR method and controlled subsequently (Papapetropoulou and Vantavakies, 1998). In another Finnish study, samplings were done on the water from seven covered swimming pools. The collected samples from five pools showed *Mycobacterium kansasii*, *Mycobacterium gordonae* and opportunistic mycobacteria (Livanainen, 1999).

Contamination with *E. coli* type O157 leads to acute intestinal infection, which could culminate in haemolytic uremic syndrome (HUS) and finally death. This infection was observed in six children living in South West London in a summer month. Three of them were hospitalized for HUS and one of them died. *E. coli* agent was isolated from the feces of five patients. All children were swimming in paddling pools and they were infected with the agent *E. coli* O157. This study indicates that even paddling pools need to be chlorinated and disinfected (Hildebrand *et al*., 1996). Another similar study showed that obstruction of chlorine feed tube and unsuitability of pool maintenance methods in Vermont has been associated with the prevalence of acute gastroenteritis in children (Zanardi Blevins *et al*., 2004). Pools used by children were mostly contaminated with fecal coliforms (Butler and Ferson, 1997). Leoni and Legnani (2001) investigated the prevalence of *Legionella* bacterium in the water of 12 swimming pools in Bologna, Italy. From each pool four samples per year (one sample per season) were taken. Of all 48 samples, two showed contamination with *Legionella*. In this survey, of 48 samples taken from warm water showers, 19 cases were positive for *Legionella pneumophila* (Leoni and Legnani, 2001). In a similar study, the microbial and physicochemical status of water from two swimming pools was tested in Tenerife, Spain. The pool which originated from seawater showed a higher microbial contamination. In samples taken from pools filled with municipal water, species of mycobacterium were isolated (Martin Delgado, 1992). Czeczelewski examined the physicochemical and bacteriological properties of water from three swimming pools, which had the best status in relation to others in the town of Biala Podlaska of Poland. It was demonstrated that with rising contamination burden, free chlorine concentration declined and in 18% of collected samples, bacterial contamination has been higher than the threshold limit (Czeczelewski, 1994).

There are 30 public pools for a population of about 1.2 million people in Shiraz. To the best of authors' knowledge, to date no systematic study has been carried out in Shiraz to assess the extent, frequency and causes of pool water contamination in order to identify and prioritize areas where preventative interventions might be usefully targeted, the current study was therefore, undertaken to address these issues.

MATERIALS AND METHODS

In this study, all public swimming pools in the town of Shiraz (52°30' N, 29°40' E) were surveyed. There were 30 pools, 20 of which were actively operating at the time of study. Of these, 19 pools were filled with municipal water and one was filled with well water. In total 199 samples were taken, 19 of which were from covered pools and 180 samples from exposed pools. Of all 199 samples, 189 were from pools filled with municipal water and the rest from the one filled with well water.

Samples were collected such that the top quarter of water bottles was empty for easy mixture of contents. Following preliminary investigations on each pool and consultation with relevant authorities, their peak hours and periodic shifts of operation for each pool was identified. This time limitation was variable for different pools and it was between 9.0 am to 10.0 pm. Samples were taken at peak times when the largest number of swimmers were present at the pool, so that samples really reflected critical conditions. Moreover, they were taken from the most congested points of the pool where the density of swimmers per unit area was more than other parts of the pool and consequently there was a larger burden of contamination at a depth of 30 cm under surface water. The quantity of pH, residual chlorine and temperature on site was determined. The samples were maintained at 4°C and transferred to laboratory for routine microbial test.

In order to measure residual chlorine and pH, the Merck standard kit (number: 11134) was used. Residual chlorine was measured with orthotolidine arsenite (OTA) method. Sterile bottles with a volume of 250 or 500 mL were used for doing microbial test. Sampling was carried out over a three months period (July-September) in the summer of the year 2000. In all cases, the sample volume was more than 100 mL. A few crystals of sodium thiosulphate ($S_2O_3Na_2$. $5H_2O$) was added to the collected sample to neutralize residual chlorine.

In order to estimate the number of coliforms, the multiple tube fermentation method and the table of most probable number (MPN) have been used (APHA, 1995). The method used for diagnosis, presence and density estimate of *Pseudomonas aeroginosa* was membrane filter technique with the application of MP-A agar culture medium. In this method, 500 ml of pool water was passed through a sterile membrane filter. This filter was then put on the above-mentioned culture medium and the medium-bearing plate was laid upside down in an incubator at 41.5±0.5°C temperature for 72 h. In order to differentiate between different streptococci groups, the multiple tube method and azide dextrose broth culture medium were used. The positive samples were then transferred to PSE-agar medium for confirmation test.

Laboratory tests, culture media in use and other conditions of tests such as cell count based on isolation and identification method as well as coliforms count were carried out in concordance with the guidelines of the Institute of Standards and Industrial Research of Iran on standard methods for water and wastewater tests (APHA, 1995; ISIRI, 1998).

Statistical data were analyzed with student's t-test and chi-square test.

RESULTS

Research data indicated that the water of the pool filled with well water was replaced once every 7-15 days and those filled with municipal water once every 30-45 days. The mean swimmers density at pools estimated to be 0.8 person m^{-2}, which of course varied from one pool to another. Table 1 depicts the contamination status of water from exposed and covered pools to various microbial germs on the basis of the pool water feeding source.

As it can be observed from Table 1, 51.3, 16.6, 11 and 7% of the total water samples were contaminated with *Pseudomonas*, *E. coli*, coliforms and streptococci, respectively. In this regard, 26.3% of samples collected

Table 1: The bacteriological characteristics of swimming pool's water

Bacteria	Contamination	Covered pools (No.)	(%)	Exposed pools (No.)	(%)	Well water (No.)	(%)	Tap water (No.)	(%)	Total (No.)	(%)
E. coli	Negative	19	100.0	147	81.7	9	90	157	83.1	166	83.4
	Positive	0	0.0	33	18.3	1	10	32	16.9	33	16.6
Pseudomonas	Negative	14	73.7	83	46.1	8	80	89	47.1	97	48.7
	Positive	5	26.3	97	53.9	2	20	100	52.9	102	51.3
Streptococci	Negative	19	100.0	166	92.2	9	90	176	93.1	185	93.0
	Positive	0	0.0	14	7.8	1	10	13	6.9	14	7.0
Coliforms	Negative	19	100.0	158	87.8	9	90	168	88.9	177	89.0
	Positive	0	0.0	22	12.2	1	10	21	11.1	22	11.0

Table 2: The physicochemical properties of swimming pools' water in Shiraz differentiated on positive and negative bacterial samples

Bacteria	Outcome (threshold) per 100 mL	Mean pH	Mean residual chlorine mg L^{-1}	Mean temperature (°C)	Hypochlorous acid (%)	Hypochlorite ion (%)	Mean hypochlorite Ion (mg L^{-1})	Mean hypochlorous acid (mg L^{-1})
E. coli	Negative (<100)	7.17	0.85	27.00	57	43	0.365	0.485
	Positive (>100)	7.39	0.18	26.40	36	64	0.115	0.065
Pseudomonas	Negative (<100)	7.16	1.052	27.02	57	43	0.452	0.600
	Positive (>100)	7.25	0.45	26.60	51	49	0.218	0.227
	Zero	7.21	0.97	27.08	57	43	0.417	0.553
Streptococci	Negative (<100)	7.1	0.24	26.20	64	36	0.087	0.154
	Positive (>100)	7.44	0.10	26.76	46	54	0.054	0.046
	Zero	7.21	1.03	27.16	57	43	0.443	0.587
Coliforms	Negative (<460)	7.11	0.38	26.31	64	36	0.137	0.243
	Positive (>460)	7.40	0.15	26.61	46	54	0.081	0.069

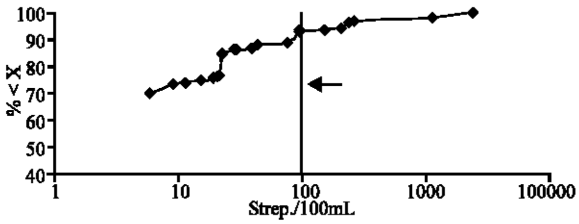

Fig. 2: The cumulative frequency of pools' contamination with streptococci

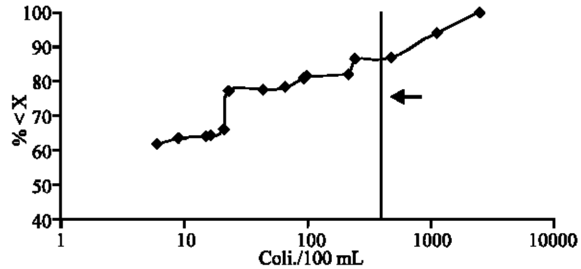

Fig. 3: The cumulative frequency of pools' contamination with coliforms

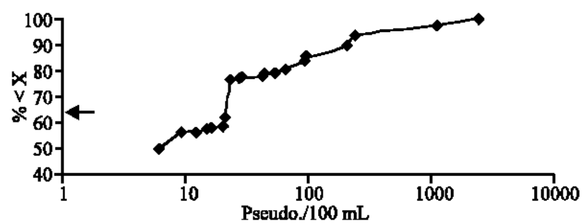

Fig. 4: The cumulative frequency of pools' contamination with pseudomonas

from covered pools and 53.9% of samples from exposed pools were contaminated with *Pseudomonas* and the statistical difference between these two sources was significant ($p<0.02$).

The results of measurements for parameters like pH, temperature, residual chlorine, etc. differentiated for positive and negative microbial tests are shown in Table 2. According to this table, the level of residual chlorine in water samples contaminated with *Pseudomonas* was 0.45 mg L^{-1} and in non-contaminated samples was 1.052 mg L^{-1}, the difference between these two values being statistically significant ($p<0.02$).

The cumulative frequencies of microbial parameters of swimming pools are exhibited in Fig. 2-4.

DISCUSSION

In all samples, the amount of residual chlorine of swimming pools water, from which the positive microbial samples were isolated, was less than the cases from which the negative microbial samples was reported. The increase in pools water pH causes a lower percentage of residual chlorine transform into hypochlorous acid and thus chlorine disinfection power reduces. In view of the fact that the extent of pools water temperature variation in this study has not been vast, so the effect of temperature on hypochlorous acid ionization dissociation coefficient and its change into hypochlorite ion can be assumed to be constant in all samples and thus could be ignored. Considering figure 1, the percentage distribution curve of HOCl and OCl^- at the pH range of 7-8 has the sharpest slope, so in practice, with a minimal drop in pH, the HOCl percentage could be elevated excessively and so raise the chlorine disinfection efficiency (Leoni and Legnani, 2001).

The mean residual chlorine of water for *Pseudomonas*-positive samples (0.4 mg L^{-1}) was less than negative ones (1.052 mg L^{-1}) and the difference between these two values was statistically significant ($p<0.02$). This finding pertains to the fact that reduction in water residual chlorine could bring about the opportunity for increase in microorganisms like *Pseudomonas*. It is interesting to note that this bacteria has exhibited a higher resistance to residual chlorine (up to 0.45 mg L^{-1}) with respect to other surveyed microorganisms. In addition, the mean residual chlorine in *E. coli*-contaminated samples was significantly different ($p<0.01$) from non-contaminated pools. In general, these observations were completely consistent with those of Czeczelewski who showed that the cause of bacterial contamination of pools water was insufficiency in the concentration of disinfectant (Czeczelewski, 1994). The fall in residual chlorine from 0.85 to 0.18 mg L^{-1} has increased to a large extent the *E. coli*-positive samples and this reflects the high susceptibility of *E. coli* to chlorine.

The mean residual chlorine in streptococci-negative samples has been 0.24 and in positives 0.1 mg L^{-1}. As for other microorganisms, the fall in residual chlorine could have an effective role in the rise of streptococci count. This situation was the same in the case of coliforms; such that the difference in mean residual chlorine within positive and negative samples was statistically significant ($p<0.006$). These results corroborate well with those of previous reports (Kebabjian, 1995; Hildebrand *et al*., 1996) in which the mortality of all bacteria in the presence of 1.0 mg L^{-1} residual chlorine in water was evident. This idea is also important since the amount of residual chlorine in water of coliform-contaminated pools has been less than the standard recommended limit of 1.0 mg L^{-1} for summer season (APHA, 1998), thus the availability of positive samples in the presence of tiny amounts of residual chlorine in this study is not unexpected. The insufficiency of the quantity of residual chlorine in pools water not only paves the way for the growth of pathogenic and non-pathogenic bacteria in pools water, but also it affects the growth of opportunistic saprophytic fungi and yeasts in pools water (Shadzi *et al.*, 1993). In crowded pools (about 500 users per day), with low residual chlorine (0.704 mg L^{-1}), fungal contamination has been more prevalent. Conversely, the prevalence of fungal contamination was intensively reduced with a fall in pool users (350-400) and a rise (2.13 mg L^{-1}) in residual chlorine (Shadzi *et al.*, 1993). In another similar study carried out by Nonbakhsh (2002) in the town of Oroumyieh, northwest Iran, four active pools were surveyed for parasitic and fungal contamination. The residual chlorine concentration of these pools varied between 0-1.2 mg L^{-1} (mean=0.6 mg L^{-1}). From 384 collected samples, 12% were positive for fungal contamination. The most prevalent fungi were: *Aspergillus*, *Candida* and dermatophytic fungi; *Mucor*, *Fuma*, *Penicillium*, *Exophiala* and species of *Rhizopus*. The low residual chlorine concentration and high number of pool users are expressed as the main causes of pools contamination.

The data presented in this study demonstrated that the source of water used in filling pools is counted as a main factor in their contamination. The study done by Martin Delgado confirms this point (Martin Delgado, 1992).

In conclusion, the observations that public swimming pools of Shiraz, apart from a few exceptions, are contaminated with different microbial organisms on the one hand; and high number of pool users on the other hand, call for more strict surveillance measures from responsible health authorities for protection of swimmers' health.

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